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Technical Brief

Lateral Flow Devices to Rapidly Determine Levels of Stable *Botrytis* Antigens in Table and Dessert Wines

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Abstract: Two commercially available *Botrytis* Lateral Flow Devices (B-LFDs) (immunochromatographic devices), one from EnviroLogix, Maine, USA the other from Forsite Diagnostics,York, UK were tested and compared for their ability to detect and quantify levels of a highly stable *Botrytis* antigen in table wines and dessert wines. Table wines were diluted 1:40 and dessert wines 1:500 in phosphate buffered saline plus Tween 20 (0.05% v/v) (PBST). Results from both types of devices were comparable and repeatability was good. This study shows that *Botrytis* Lateral Flow Devices could provide a useful tool for wine makers interested in relating levels of *Botrytis* antigens in table and dessert wines to sensory properties.

Key words: antigens, *Botrytis*, grey mould, Lateral Flow devices, noble rot, table and dessert wines

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Introduction

It is well recognized that *Botrytis cinerea* is the main cause of bunch rot of grape berries in temperate climates (Marois et al. 1993, Slomczynski et al. 1995). Bunch rot is undesirable for production of table wines because it is associated with off-flavours and unpleasant aromas in finished wines and loss of colour in red wines. In contrast, the presence of *B. cinerea* infections in late harvest grape berries is desirable because *Botrytis* is believed to be responsible for the desirable aromas and flavours (Sarrazin et al. 2007, Sivertsen et al. 2005). However, in both cases, any study of the relationship between levels of *Botrytis* in wines and sensory properties of wines is difficult because there has been no easy way to measure levels of *Botrytis* antigens in finished wines. The use of rapid, user-friendly, immunodiagnostic Lateral-Flow Devices offers an easy method to detect fungal antigens (Dewey et al. 2008). The advantages of LFDs over other immunological methods, such as microtitre plate ELISAs (Dewey et al. 2000; Dewey 2002), and molecular tests are that they are rapid, (5-15min), can be easily operated by untrained workers and do not require laboratory facilities.

Two commercially available B-LFDs have been developed for the detection and quantification of soluble stable *Botrytis*-antigens, one produced by EnviroLogix, Portland, ME, USA and the other by Forsite Pocket Diagnostics, York, UK. Although both employ the same *Botrytis* monoclonal antibody, BC-12.CA4 (Meyer and Dewey 2000), that recognizes a constitutively produced, thermostable antigen, that is not metabolized during fermentation, there are technical differences and the performance of both tests has never been compared.

The format used by both devices is a sandwich assay in which the antigens are captured by the *Botrytis* antibody which is bound, in the EnviroLogix test to gold nanoparticles and, in the Pocket Diagnostics test to 'latex' particles. The bound conjugates are present in an absorbent pad overlaying a

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nitrocellulose membrane (Fig 1). When the test liquid is applied, the particles move along the membrane by capillary action; those carrying the *Botrytis* antigens are arrested at the test line on the membrane by a pre-printed line of *Botrytis*-antibodies. In the EnviroLogix test, the positive test line appears pink because the antibody is bound to gold particles whereas the positive test line in the Forsite Pocket Diagnostics devices appears blue because the latex-antibody conjugate is blue. Those particles to which no antigen is bound continue to move forward along the membrane and, in the case of the EnviroLogix test they are arrested at the control line, by a pre-printed line of anti-mouse antibodies, to give a second pink line. The control line in the Pocket Diagnostics test is formed in a different way. Blue latex particles, coated with non-specific rabbit-antibodies, are mixed with the murine *Botrytis*-bound latex particles in the absorbent starting pad and these, which are not arrested at the test line, are arrested at the control line by a line of anti-rabbit antibodies (Fig. 2). The differences between the two types of LFDs, apart from colour of the test and control bands, could be significant. The control line in the EnviroLogix tests is dependent on there being an excess of *Botrytis* gold-conjugates particles binding at the control line whereas the Pocket Diagnostics control line is not dependent on an excess of *Botrytis* antibody coated latex particles. These technical differences mean that, if excess antigen is present, there could be significant differences in the intensity of the control bands because the SI value is the percentage of the reflectivity of the background minus that of the test line divided by the reflectivity of the background minus that of the control line. The reflectivities of the test lines, control lines and backgrounds are measured with their respective custom-made scanners (reflectometers). The percentage of the reflectivity of the background minus that of the test line divided by the reflectivity of the control line is automatically expressed by the scanners as the Signal Intensity (SI).

The aims of this study were to compare two commercially available B-LFDs for the quantification of *Botrytis* antigens in a range of red and white table wines and dessert wines.

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Materials and Methods

Wines. All wines tested were purchased either in the UK or Australia or, in the case of the reference dessert wine Dolce, from Far Niente, California, USA. Details of grape varietal and vintage of the wines are given for red wines in Table 1, white wines in Table 2, dessert wines in Table 3. All wines were stored and tested at room temperature, precipitates were not seen in any of the red wines.

Lateral Flow Devices. B-LFD kits and their respective scanners were purchased from EnviroLogix (kit batch number 151208) (Portland, ME, USA) and from Forsite Pocket Diagnostics (kit batch number Y01 for table wines and AA01 for desert wines) (York, UK). The two different batches of Forsite Pocket Diagnostic kits differed in that AA01 had a higher concentration of the *Botrytis* antibody conjugated particles in the test pad than Y01. Unless otherwise stated, all wines were diluted into phosphate buffered saline + Tween 20 (0.05% v/v) (PBST), table wines were diluted 1:40, dessert wines 1:500. Tests with the EnviroLogix B-LFDs were done by placing the absorbent pad of the device in 400µl of diluted wine for 10 min; the absorbent pad was then removed and the device was inserted into the EnviroLogix reader (scanner) to determine the SI value. For the Forsite Pocket Diagnostics tests, 70 µl of the diluent were pipetted into the well of the device and, after 10 min, the devices were inserted into the Forsite reader and the SI value recorded. On completion the SI values of the EnviroLogix tests remained stable because the absorbent start pad is removed before the SI values are determined but the SI values of the Pocket Diagnostics tests tend to increase with time because the start pad is not removed. Therefore, care was taken to time the tests and determine the SI values in both cases after 10 min.

To determine repeatability of the devices and threshold detection levels a dilution series of the reference dessert wine Dolce (Far Niente, CA, USA, 1998 vintage), used in previous studies (Dewey et al. 2008) was tested.

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Results and Discussion

Standards and Repeatability In tests, done with both devices, on a series of dilutions of the reference dessert wine, Dolce, correlation was good, R^2 0.939, (Fig 3). The problem of repeatability was addressed in a previous publication by Dewey et al, (2008) where tests were done in triplicate on a dilution series of the dessert wine Dolce. There was little variation between replicates and so for this reason, and to reduce total costs of the project, tests on each of the experimental table and dessert wines were only done once. The threshold detections levels were similar. Both devices could detect down to 0.0125% standard in PBST but there were differences in the numerical value of the signals from the control or buffer alone, SI values for the EnviroLogix devices were zero, but for the Forsite devices they ranged from 0 to 6 (batch Y01) and from 18.6 to 20 (batch AA01).

Table wines. SI values from the tests done on 36 red and 32 white table wines and 27 dessert wines are given in Tables 1, 2, and 3, respectively. In all cases, there was good correlation between the SI values form the EnviroLogix B-LFDs and the Forsite Pocket Diagnostics B-LFDs (Figs. 4A, B and C For red wines, the R^2 value was 0.867 for white wines 0.7456 and 0.849 for dessert wines. The SI values from tests on red wines by the EnviroLogix B-LFDs ranged from 0 to 48 and from 3.3 to 36 by the Forsite Diagnostics B-LFDs. In tests on white wines, SI values ranged from 0 to 44 with the EnviroLogix B-LFDs and from 3.3 to 36 with the Forsite Pocket Diagnostics LFDs. The range in SI values from tests with the EnvioLogix B-LFDs was greater than those with the Forsite Pocket Diagnostics LFDs; this, in the case of the high-end results, probably reflects the difference in the capture antibodies used for the control lines. At high concentrations of antigen, the *Botrytis* binding sites on the antibody-coated particles in the test pad of both devices become saturated. This does not affect the intensity of the control line in the Forsite devices because the formation of the control line is independent of the level of antigen

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117 binding. But, in the EnviroLogix devices the control line is dependent upon there being an excess of free,
118 unbound *Botrytis* antibody-coated nanogold particles. Therefore, when there are fewer *Botrytis*-free
119 antibody bound particles available to travel to the control line the intensity of the control line is lower. In
120 both cases, because the SI value is calculated from the percentage difference between the reflectance of
121 the background minus the test line divided by the reflectance of the background minus the control line, a
122 weaker control line would give higher SI values.

123 **Dessert wines**. The SI values using the Forsite B-LFD (batch AA01) were much higher than
124 those from Envirologix B-LFDs. Although the values were higher (15 to 134) they correlated well with
125 the SI values from the EnviroLogix B-LFDs (0-50) giving an R^2 value of 0.849 (Fig. 4C).

126 **Applications.** Both devices are simple and easy to use**.** The Forsite Pocket Diagnostics devices
127 and their custom made reader (scanner) are designed to be used in the field. Their devices, which are
128 encased in plastic, are more robust but more expensive.

129 Pilot studies, not reported here, have shown that the level of *Botrytis*-antigens in grape juice
130 remains constant throughout fermentation irrespective of grape varietal. These devices should therefore,
131 provide useful tools for relating levels of *Botrytis* antigens to the sensory properties of wines such as taste
132 and aroma and could also be of particular value for sparkling wine production where proteases produced
133 by *Botrytis* are known to affect the formation and retention of bubbles (Marchal et al. 2001, Cilindre et al.
134 2007).

135 We did not attempt to relate the SI values from tests on finished wines to levels of rot in the
136 vineyard. Previous studies have shown that the *Botrytis*-SI values correlate well with tests on juice from
137 hand-picked, hand-sorted, berries but not with visual estimates of machine-picked grapes (Dewey et al.
138 2008). In the latter, heavily-infected berries are often not counted because they are easily squashed

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during transit. On-site field estimates of rot are also problematic because they are totally dependent on the method of sampling and the variations found within different climatic regions in vineyards. We found that, in tests with juices, diluted 1:40 in PBST, from hand-picked, hand-sorted grape berries (Chardonnay) with 0.6, 1.3, 2.5, 5.0 and 10.0% *Botrytis*- rot (determined on the basis of incidence, not weight) that SI values with the EnviroLogix B-LFDs were 10.5, 16.7, 26.2, 34.6 and 39.7 respectively (Dewey et al., 2008). Thus, B-LFD tests on red and white table wines with SI values greater than 35 indicate that the wines were made from grape berries with 5% or more average level of *Botrytis* rot. However, the value of the *Botrytis* LFDs is not in determining estimates of rot in the field but in providing a means of measuring the levels of *Botrytis* antigens throughout the fermentation process and in finished wines.

Conclusions

Both the EnviroLogix and the Forsite Pocket Diagnostics *Botrytis* LFDs are useful tools for rapidly determining levels of *Botrytis* antigens in wines. They provide an easy mechanism for wine makers interested in studying the relationship between of the levels of *Botrytis* antigens and the aroma and flavours of wines. Results from both devices were comparable. The Forsite Pocket Diagnostics B-LFDs and their custom-made reader are designed for field use; they are more expensive than the EnviroLogix B-LFDs but, because they are housed in plastic they have the advantage of being more robust.

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Table 1 Red wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. SI = signal intensity. Wines were diluted 1/40 in PBST prior to analysis. Each wine was only tested once.

Variety	Origin	Vintage	SI EL	SI FPD
Barbera	Italy	2007	27	18.1
Cab Sauv	Australia	2006	0	5.2
Cab Sauv	Australia	2009	0	4.8
Cab Sauv / Merlot	Australia	2008	0	6.3
Cab Sauv / Merlot	Chile	2009	5	5.9
Cab Sauv / Merlot	France	2005	10	6.3
Carmenere	Chile	Unknown	9	6.8
Grenache / Tempranillo	Spain	2009	18	10.3
Malbec	Argentina	2008	5	5.6
Malbec	Argentina	Unknown	14	9.1
Merlot	Chile	2009	7	3.3
Merlot / Cab Sauv	France	2007	28	15.3
Monastrell	Spain	2003	18	10.6
Monastrell	Spain	2005	37	18.5
Pinot noir	Argentina	2008	40	24.3
Pinot noir	Australia	2005	23	13.4
Pinot noir	Australia	2008	0	5.2
Pinot noir	Australia	2006	0	6.6
Rioja	Spain	2003	32	20.6
Rioja	Spain	2005	35	19
Rioja	Spain	2007	35	16.6
Rioja	Spain	2008	36	17.6
Shiraz	Australia	2008	0	3.4
Shiraz	Australia	2009	4	4.9
Syrah	France	2007	30	17.9
Unknown	France -Beaujolais	2007	35	15.9
Unknown	France -Bordeaux	1988	10	6.8
Unknown	France -Bordeaux	1988	48	35.5
Unknown	France -Bordeaux	1998	48	34.3
Unknown	France -Bordeaux	2005	5	3.2
Unknown	France -Bordeaux	2007	29	17
Unknown	France -Cotes du Rhone	2001	8	6.9
Unknown	Italy -Chianti	1998	38	20.1
Unknown	Italy -Chianti	2006	22	10.8
Unknown	Portugal -Alentejo	2008	5	6.2
Unknown	Spain -Madeira	1981	29	11.3

Cab Sauv = Cabernet Sauvignon.

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Table 2 White wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. SI = signal intensity. Wines were diluted 1/40 in PBST prior to analysis. Each wine was only tested once.

Variety	Origin	Vintage	SI EL	SI FPD
Chardonnay	Australia	2007	13	7.2
Chardonnay	Australia	2008	0	3.5
Chardonnay	Australia	2008	7	7.8
Chardonnay	Australia	2009	0	7.1
Chardonnay	Australia	2009	0	4.0
Chardonnay	Australia	2008	4	10.0
Chardonnay	Chile	2009	8	4.7
Chardonnay	France	2007	41	26.6
Chardonnay	France	2008	30	16.4
Chardonnay	France	2008	44	28.5
Chardonnay	S. Africa	2008	8	7.7
Chardonnay	S. Africa	2009	6	5.5
Chardonnay	S. Africa	2009	9	7.1
Chardonnay	Australia	2008	43	29.2
Chardonnay/Viognier	France	2009	0	14.2
Gewurztraminer	Chile	2008	9	0.7
Muscat	France	2008	29	8.2
Petit Chablis	France	2008	38	22.4
Pinot Grigio	Italy	2009	15	8.6
Pinot Grigio	Italy	2009	16	7.7
Pinot Grigio	NZ	2008	0	7.2
Pinot Grigio	Italy	2009	11	9.1
Pouilly Fume	France	2008	29	14.9
Riesling	Australia	2008	2	5.6
Riesling	Germany	2007	32	18.7
Riesling	Germany	2007	28	20.4
Riesling	NZ	2008	34	23.4
Sancerre	French	2007	29	14.0
Sauvignon Blanc	Chile	2009	16	6.7
Sem. / Sauv. Blanc	Australia	2009	11	3.6
UWB	France	2008	9	9.4
Viognier	Argentina	2009	7	5.4

UWB = Unknown white blend

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Table 3 Dessert wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. Wines were diluted 1/500 in PBST prior to analysis. SI = signal intensity. Bottle rep = bottle replicates of the same wine/vintage. Each bottle was only tested once. All wines of Australian origin were made with Semillon grapes while wines from France were made with an unknown blend of Semillon and Sauvignon Blanc.

Wine	Bottle rep	Origin	Vintage	SI EL	FPD
1	1	Australia	1984	45	100
2	1	Australia	1985	40	82
3	1	Australia	1995	45	96
4	1	Australia	2000	41	106
5	1	Australia	2002	34	79
6	1	Australia	2004	25	71
6	2	Australia	2004	22	61
6	3	Australia	2004	25	79
6	4	Australia	2004	27	66
7	1	Australia	2004	30	57
7	2	Australia	2004	29	59
7	3	Australia	2004	30	68
8	1	Australia	2005	14	32
9	1	Australia	2006	0	15
9	2	Australia	2006	0	17
9	3	Australia	2006	0	14
10	1	Australia	2006	24	86
10	2	Australia	2006	25	57
10	3	Australia	2006	22	44
11	1	Australia	2006	5	32
11	2	Australia	2006	5	34
11	3	Australia	2006	6	35
12	1	Australia	2006	35	62
12	2	Australia	2006	36	88
12	3	Australia	2006	27	68
13	1	Australia	2006	23	85
13	2	Australia	2006	16	36
13	3	Australia	2006	16	42
13	4	Australia	2006	24	46
14	1	Australia	2007	13	44
14	2	Australia	2007	17	53
14	3	Australia	2007	16	44
15	1	Australia	2007	33	75
15	2	Australia	2007	29	75
15	3	Australia	2007	33	92
16	1	Australia	2008	42	100
16	2	Australia	2008	47	98
16	3	Australia	2008	47	126
17	1	Australia	2008	47	89
17	2	Australia	2008	46	115
18	1	Australia	2008	36	72
18	2	Australia	2008	33	94
18	3	Australia	2008	36	73
18	4	Australia	2008	35	74

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Table 3 continued

Wine	Bottle rep	Origin	Vintage	SI EL	FPD
19	1	Australia	2009	42	97
19	2	Australia	2009	44	104
19	3	Australia	2009	44	114
20	1	Australia	unknown	46	104
21	1	Australia	unknown	50	118
22	1	Australia	unknown	49	134
23	1	Australia	unknown	36	86
24	1	France	2001	13	47
24	2	France	2001	15	53
24	3	France	2001	17	34
25	1	France	2004	23	32
25	2	France	2004	25	64
25	3	France	2004	25	61
26	1	France	2005	17	48
26	2	France	2005	16	35
26	3	France	2005	15	35
27	1	France	unknown	18	61

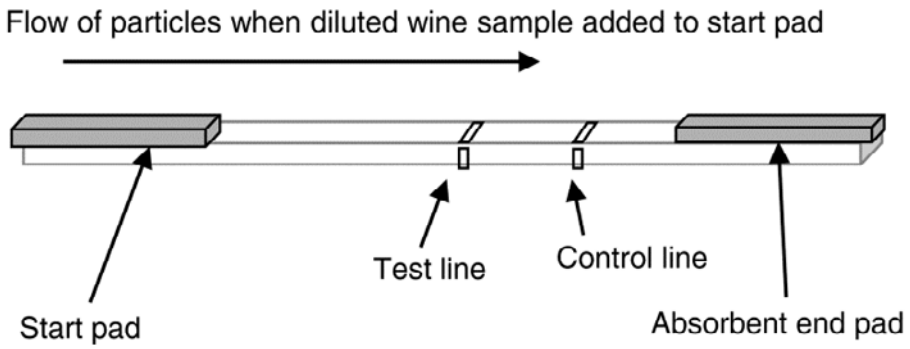

Figure 1 Diagram of Lateral Flow Device. The absorbent start pad in EnviroLogix devices contains *Botrytis* antibodies bound to nanogold particles and the control line is a pre-printed line of anti-mouse antibodies. In the Forsite Diagnostics devices the absorbent start pad contains two sets of particles one to which *Botrytis* antibodies are bound and the other to which non-specific rabbit antibodies are bound; the control line is composed of a pre-printed line non-specific anti-rabbit antibodies.

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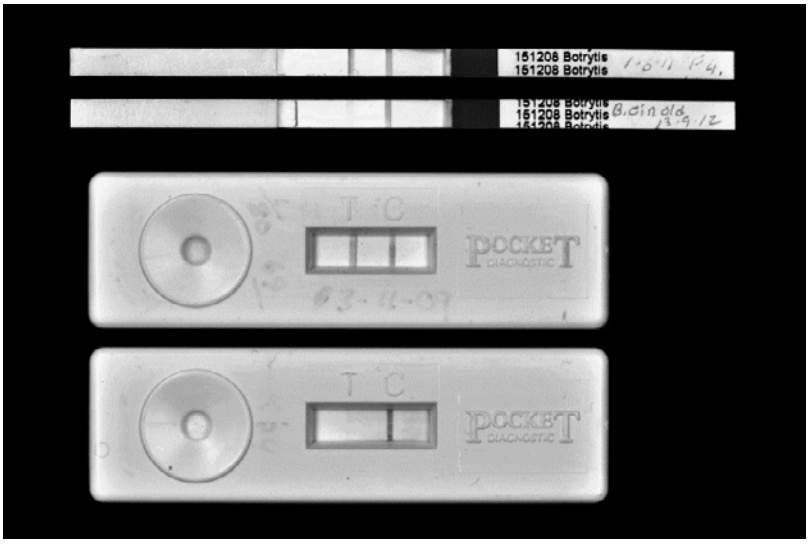

Figure 2 Completed Lateral-Flow tests, (a) EnviroLogix tests, (b) Forsite-Pocket Diagnostics tests: + positive test result, - negative test result.

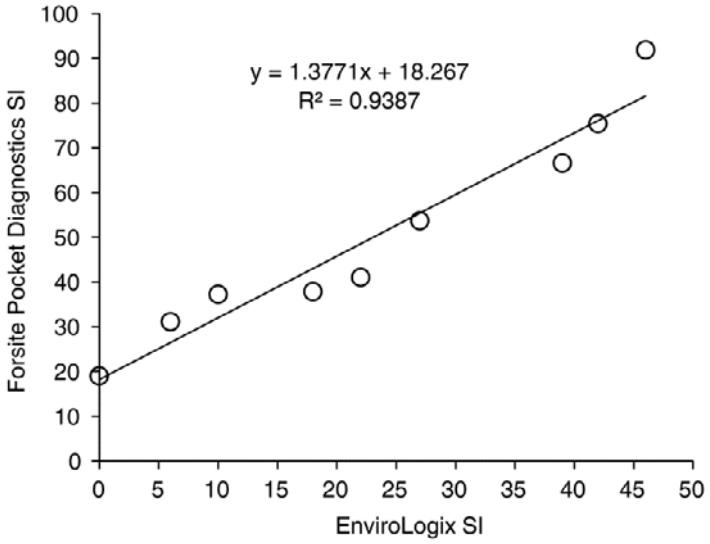

Figure 3 Comparison of the EnviroLogix B-LFDs with the Forsite Pocket Diagnostics B-LFDs (batch U05) for the quantification of *Botrytis* antigens in a dilution series (0.0 - 1%) of the reference dessert wine, Dolce, in PBST, SI= Signal intensity.

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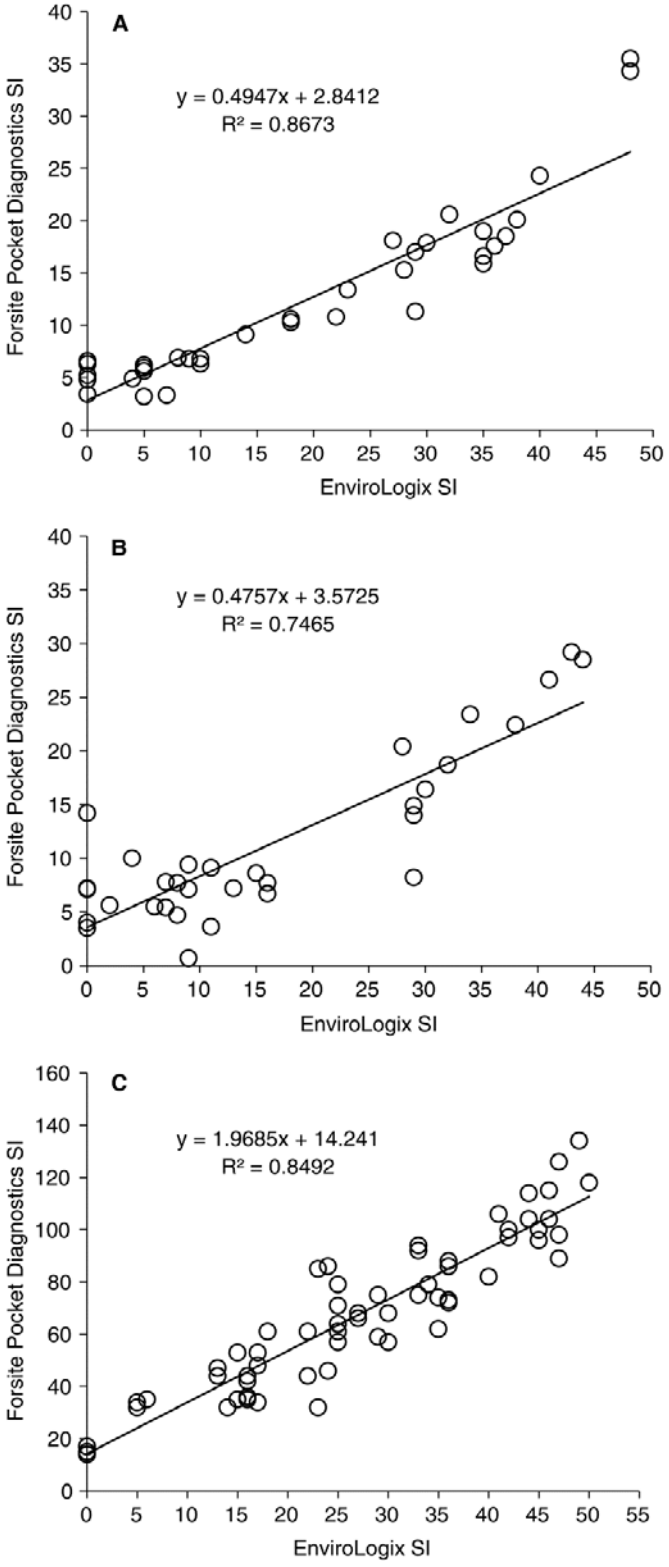

Figure 4 Comparison of the EnviroLogix B-LFD with the Forsite Pocket Diagnostics B-LFD for the quantification of *Botrytis* antigens in red wines (**A**), white wines (**B**), and a series of dessert wines from Australia and France (**C**). SI= Signal intensity.